BINDING OF THE CHEMICAL CARCINOGEN, *p*-DIMETHYLAMINOAZOBENZENE, BY HUMAN PLASMA LOW DENSITY LIPOPROTEINS

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1. Introduction

Human plasma lipoproteins are lipid–protein complexes that transport water-insoluble lipids in the circulation and regulate lipid synthesis and catabolism [1–3]. For many years, the plasma lipoproteins have attracted the attention of investigators interested in lipid transport [4,5] and cardiovascular disease. However, in addition to their involvement in lipid transport and metabolism, other roles for the plasma lipoproteins have been recognized recently, including inhibition of lymphocyte proliferation [6] and maintenance of immunologic homeostasis [7]. Plasma lipoproteins transport not only lipids but other lipophilic materials such as the drug reserpine [8], β-carotene and lycopene. α-Tocopherol and dolichol [9] are specifically associated with human plasma low density lipoproteins (LDL) and high density lipoproteins (HDL), respectively. Benditt [10] has suggested that two potential mutagens, benzo[a]pyrene (BP) and methylcholanthrene, are also carried by the lipoproteins. It is believed that the blood is the main transport mechanism in cases of chemical carcinogens or drug action where systemic effects are observed [11].

p-Dimethylaminoazobenzene (DAB), also known as butter yellow, is included in category 1 on the tentative carcinogen list issued by the Occupational Safety and Health Administration, USA [12]. DAB-induced carcinogenesis in animals has been studied extensively [13,14]. We report here that this water-insoluble carcinogen associates primarily with the human plasma low density lipoproteins (LDL).

2. Materials and methods

The present study was performed with unpooled plasma obtained from normal healthy male and female subjects. Lipoproteins were isolated by the method of Rudel et al. [15]. The solvent density of human plasma was increased to 1.225 g/ml by the addition of solid KBr and total lipoproteins were isolated by ultracentrifugation with a Beckman 60 Ti rotor at 45 000 rev./min and 10°C for 48 h. After dialysis against 0.01 M Tris, 0.1 M NaCl, 0.01% EDTA, 0.02% sodium azide, pH 7.4, the lipoproteins (1 ml) were chromatographed on a BioGel A-15 m (200–400 mesh) agarose column (1.6 × 90 cm) which was eluted at 8 ml/h. *p*-Dimethylaminoazobenzene[phenyl-^{14}C] (^{14}C-DAB) was obtained from New England Nuclear with a specific activity of 4.5 mCi/mM and purity >99%. Albumin (fraction V) was from Miles Laboratories, Elkhart, Indiana, USA. Incorporation of ^{14}C-DAB into whole blood, human plasma lipoproteins, and plasma albumin was performed as follows. About 0.5 μCi of ^{14}C-DAB was dissolved in 1 ml of benzene and evaporated to dryness in a test tube with ultrapure nitrogen. 1 ml of whole blood, plasma lipoprotein solution, or albumin solution was added and then incubated with stirring at 37°C for 6 h. In the DAB binding capacity experiments which did not involve blood cells, undissolved DAB was removed by centrifugation at 4000 × *g* for 10 min. The transparent lipoprotein mixture solution was resolved into its component lipoprotein classes by gel filtration as described above.

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3. Results and discussion

Incubation of ^{14}C-DAB with 1 ml of whole blood was attended by the distribution of 15% of the radioactivity into cellular components, the remainder being bound to the lipoproteins and lipid-free plasma proteins. In order to determine the distribution of the carcinogen among the lipoproteins, the three principal classes (VLDL, LDL, HDL) were separated by agarose gel filtration chromatography. Figure 1A illustrates the quality of the separation of these three particle populations. Although the extinction coefficient at 280 nm for each lipoprotein type is not the same, thereby preventing a direct comparison of the relative amounts of each, A_{280} measurements do permit an estimate of the amount of material present. Incubation of a 1 ml aliquot of the same plasma as used in fig.1A with ^{14}C-DAB resulted in the distribution of radioactivity among the lipoproteins as illustrated in

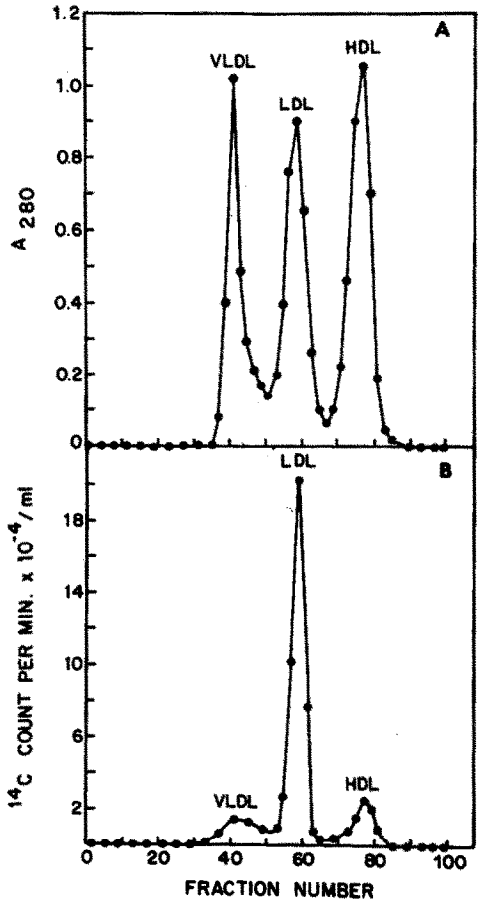

Fig.1. (A) Elution profile of plasma lipoproteins isolated by ultracentrifugation at d = 1.225 g/ml and separated on a BioGel A-15m agarose column (1.5 × 95 cm) at 24°C. A 1.0 ml sample volume (26 mg of lipoprotein) was eluted with 0.01 M Tris–HCl buffer, pH 7.4, containing 0.10 M NaCl, 0.01% EDTA and 0.02% NaN_3. (B) Distribution of ^{14}C-DAB (cpm/fraction) among a mixture of VLDL, LDL, and HDL. The lipoproteins (26 mg total in 1 ml) were incubated with 0.5 μCi of ^{14}C-DAB and then separated by gel filtration as described in the text and fig.1A.

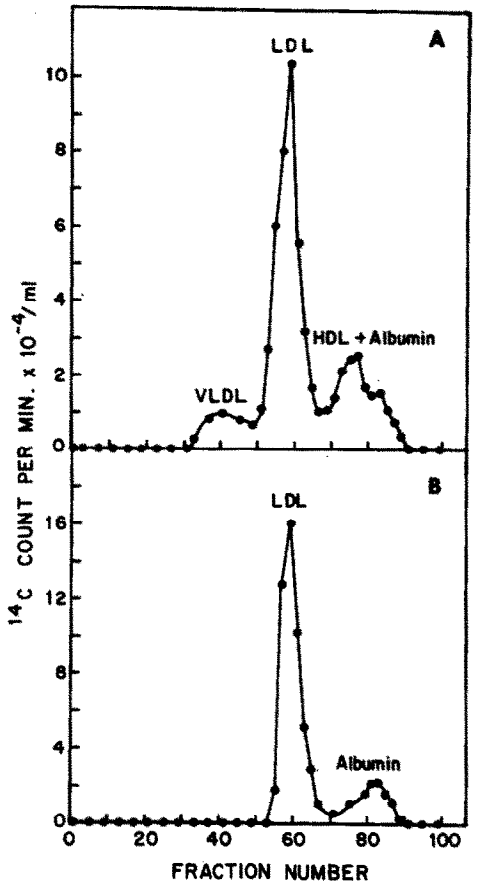

Fig.2. (A) Elution profile indicating the distribution of ^{14}C-DAB (cpm/fraction) among the lipoproteins in the presence of an exogenously added, physiological ratio of human serum albumin. Chromatographic conditions were identical to those described in fig.1A. (B) Elution profile illustrating the distribution of ^{14}C-DAB between LDL (13.2 mg) and HSA (150 mg) which were present in 1 ml buffer and were at their normal plasma ratio. The LDL is present at a concentration comparable to that of the experiment shown in fig.1B.

fig.1B. About 77% of the incorporated ^{14}C-DAB was associated with LDL, while 10% and 13% were associated with VLDL and HDL, respectively. When ^{14}C-DAB was incubated with the total plasma lipoprotein mixture and a physiologically equivalent amount of plasma albumin (150 mg), the distribution of ^{14}C-DAB among the lipoproteins was only modestly altered (65% in LDL, 9% in VLDL, and 26% in HDL/albumin) (fig.2A). The 12% decrease in DAB binding to LDL observed when albumin was in the lipoprotein mixture prompted us to more closely examine the effect of albumin on DAB binding to LDL. Incubation of ^{14}C-DAB (at a subsaturating level) with LDL and albumin present at a normal physiological ratio resulted in the distribution of 88% of the radioactivity into the LDL and 12% into the albumin fraction (fig.2B).

To insure that the binding of DAB to LDL was complete under the conditions employed, the time-dependence of this binding was examined. A maximum of 0.25 mg DAB was bound to 2.6 mg LDL at 37°C. Greater than 50% maximal binding was observed within the first 10 min, while about 95% binding occurred within the first 4 h (fig.3).

From the above studies it is clear that in mixtures of lipoproteins either containing or excluding albumin, DAB at subsaturating levels binds predominantly to LDL. In order to evaluate the possible influence on DAB binding to a lipoprotein by one or more other lipoprotein types, the DAB binding capacity of each individual lipoprotein class and albumin was determined separately (fig.4). These experiments were conducted at physiological concentrations. LDL, normally present at about 2.6 mg/ml, bound 0.24 mg DAB which was about three times greater than that bound by VLDL and four times greater than that bound by HDL at their respective plasma concentrations.

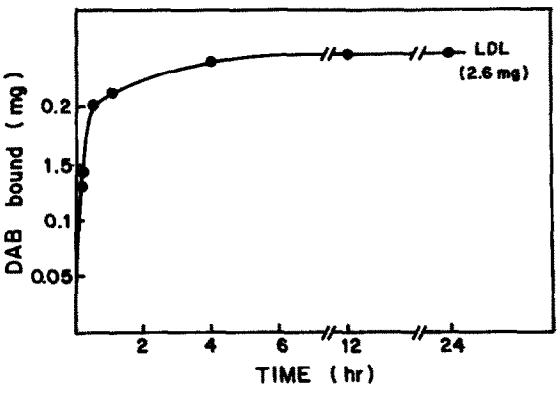

Fig.3. The rate of DAB saturation binding to LDL. The experimental details are the same as in fig.4, except that a constant saturating level of DAB (2.5 mg) was used.

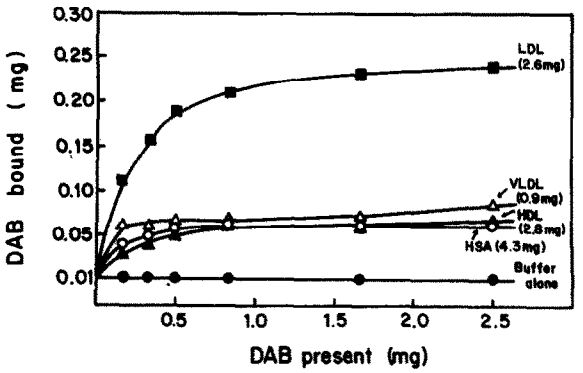

Fig.4. DAB binding capacity of VLDL, LDL, HDL and HSA. Each component, at its normal plasma concentration, was incubated with increasing amounts of ^{14}C-DAB at 37°C for 12 h. Undissolved DAB was removed by centrifugation (4000 rev./min, 10 min). An aliquot of the supernatant was used for quantitation. The figure indicates the total DAB (mg) found in the supernatant at each concentration of DAB. No additional DAB was found in the supernatant with extended time (up to 48 h).

In normal male (30–39 years) plasma as used in this study, the mean VLDL, LDL, and HDL concentrations are 90, 262, and 283 mg%, respectively [16]. Since these particles contain about 90, 80, and 50% lipid by weight, the relative percentage of lipid transported by each type is approximately 19, 48, and 33%, respectively. Hence, the distribution of a major portion of DAB into the LDL is understandable, at least in part, in terms of the fraction of total lipids transported by these lipoproteins.

The above argument predicts that altering the relative abundance of lipoprotein classes will affect the distribution of DAB among them if it undergoes sufficiently rapid chemical exchange. Cholesterol and phospholipid are known to exchange or transfer among lipoproteins and between the various blood components [1,20]. Accordingly, the transfer of DAB under non-physiological lipoprotein ratio conditions was tested. At equal protein concentrations (1.0 mg/ml) for each lipoprotein class, 53% of the ^{14}C-DAB originally associated with LDL transferred to VLDL,

10% to HDL, while <5% transferred to albumin which was present at 10 times the apolipoprotein concentration. The high efficiency of ^{14}C-DAB transfer from LDL to VLDL is similar to that found for benzo[a]pyrene (BP) by Shu and Nichols [17]+. Like DAB, BP was found to bind predominantly to LDL. When BP was incubated with the plasma from a hypertriglyceridemic patient, the VLDL became its primary carrier.

The observation that 17–29% more DAB distributes into LDL than is predicted suggests that the lipoprotein may possess unique structural features [3,17–19] which favor DAB binding.

Although our study does not establish the actual binding site(s) for the DAB, the extremely apolar lipid core of VLDL and LDL [3] would be a logical candidate for this function. Indeed, the lipophilicity (decimal logarithm of the partition coefficient) of DAB was determined for the octanol–water system and found to be 4.26 ± 0.11. This relatively high lipophilicity, compared to a number of sterols (e.g. progesterone and testosterone) [21], strongly suggests that it is indeed the hydrophobic core of these lipoproteins where DAB is bound.

The binding of chemical carcinogens or drugs at non-active sites such as plasma albumin and lipoproteins has important biological implications since these entities may influence access to sites of biotransformation, action, and excretion. Although plasma albumin, the most abundant protein in the blood, is the major drug-binding protein [22], Gillette [23] and Chen et al. [8] have pointed out that drugs may bind to other plasma proteins. In the case of the two water-insoluble chemical carcinogens, DAB and BP, both are found associated mainly with plasma LDL, not albumin. If LDL is the major transport vehicle for water-insoluble carcinogens in vivo, the ramifications may be profound. Specific receptor sites for LDL exist in a variety of normal cells such as human fibroblasts, lymphocytes, and arterial smooth muscle cells [24]. The interaction of LDL with this specific receptor initiates a series of complex processes involving endocytosis and lysosomal degradation of the internalized LDL, with concomitant activation of acyl-coenzyme A : cholesterol acyltransferase (ACAT) and suppression of HMG-CoA reductase within cultured cells [24]. The transfer of chemical carcinogens from LDL to cells by either this process or by some other transfer mechanism might cause neoplastic transformations if the accessible cells possess the enzyme systems responsible for carcinogenic metabolic activation [13]. This process might also serve as an initiation factor in the transformation of arterial smooth muscle cells followed by a progressive lesion development with the formation of atheromata [25].

+ While this manuscript was in preparation, the report of Shu and Nichols [17] was published. Their results for the binding of benzo[a]pyrene to human plasma lipoproteins are very similar to those described here for DAB. The majority of BP was found associated with the VLDL and LDL, and the distribution of this carcinogen was found to depend on the relative abundance of the different lipoprotein classes.

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